AD_____

Award Number: DAMD17-00-1-0237

TITLE: Functional Analysis of the Transcriptional Co-Activator CBP in Wnt-signaling Dependent

PRINCIPAL INVESTIGATOR: Tso-Pang Yao, Ph.D.

CONTRACTING ORGANIZATION: Duke University Medical Center
Durham, North Carolina 27710

REPORT DATE: May 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2001	Annual (17 Apr 00 - 16 Apr 01)

4. TITLE AND SUBTITLE
Functional Analysis of the Transcriptional Co-Activator CBP in Wnt-signaling Dependent

5. FUNDING NUMBERS
DAMD17-00-1-0237

6. AUTHOR(S)
Tso-Pang Yao, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Duke University Medical Center
Durham, North Carolina 27710

E-Mail: Yao00001@mc.duke.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Wnt signaling is mediated by a multi-component cascade that relays the signal from cell membrane to nuclear TCF-β-catenin transcriptional complex and thereby, modulates gene expression. Gain of function mutations in the Wnt pathway lead to various cancers. Genetic study has implicated CREB-Binding Protein (CBP) as a negative regulator of Wnt signaling, suggesting that CBP could modulate the wnt-induced carcinogenesis. In the current study, we examined the functional relationship between CBP and Wnt-dependent transcription mediated by TCF-β-catenin. Unexpectedly, we found that CBP does not negatively regulate TCF-b-catenin-mediated transcription. Instead, CBP potentiates TCF-β-catenin transcriptional activity by physically interacting with β-catenin. Our results have two implications. First, as CBP is a known transcriptional co-activator, the β-catenin-CBP interaction provides a mechanism to explain how recruitment of β-catenin activates TCF transcriptional activity. Second, it suggests a possibility that β-catenin might function as a molecular switch that modulates the activity of CBP toward TCF-dependent transcription events. We hypothesize that in the absence of β-catenin, CBP facilitates the function of TCF as a transcriptional repressor. Upon binding to β-catenin, CBP is converted to a classical transcriptional co-activator and forms a TCF-β-catenin-CBP complex that activates wnt-dependent downstream target genes.

14. SUBJECT TERMS
wnt, TCF, CBP, acetylation, transcription

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Progress Report:

Title: Functional analysis of the transcriptional co-activator CBP in Wnt-signaling dependent mammary carcinogenesis

Introduction

The long-term objective of this proposal is to analyze the role of the transcription co-activator CBP in Wnt-signaling dependent carcinogenesis. In mouse model, wnt over-expression in mammary gland results in the development of breast carcinoma. Genetic study in Drosophila has implicated CBP as a negative regulator of TCF transcription factor, the major downstream effector of wnt signaling(1). As heterozygous mutant CBP mice develop mammary gland hyperplasia, these observations suggest that CBP might play a negative role in regulating wnt-dependent mammary gland carcinogenesis. Molecularly, TCF can function both as a transcription repressor and activator (2). In the absence of wnt, TCF actively suppresses the wnt-responsive genes. However, wnt activation leads to the formation of β-catenin –TCF complex, which functions as a transcriptional activator on wnt-responsive genes. In our proposal, we hypothesized that CBP negatively regulates wnt-signaling by either dominantly suppressing TCF-β-catenin -dependent transcriptional activation or by promoting the transcriptional repressor function of TCF. Some of these issues were addressed in the first 12 months of the award period.

Body

Thc first phase of the work is trying to accomplish the Technical Objective number 1, which aims at "establishing whether CBP regulates the transcriptional activity of TCF-β-catenin complex". As it will be discussed further, we have now firmly established the functional relationship between wnt-β-catenin-TCF dependent transcription and CBP/p300 family members.

Based on our hypothesis that CBP/p300 might negatively regulate TCF4 dependent transcription, we first test whether over-expression of CBP/p300 can suppress TCF-β-catenin-dependent transcription. As shown in Figure1, expression of TCF4 and -β-catenin together results in an increase of TCF-reporter gene. However, co-expression of CBP does not suppress this activity. In contrast, CBP increases the reporter activity in a dose-dependent manner. This result demonstrates that CBP does not repress TCF4--β-catenin transcription activity; rather it functions as a co-activator in this context. To examine whether CBP has similar effect on endogenous TCF-β-catenin activity, we analyze SW480, which contains constitute active-TCF-β-catenin complex due to a mutation on APC tumor suppressor. As shown in Figure 1B, CBP can similarly enhance the transfected TCF reporter activity in Sw480, supporting that CBP can enhance the activity of TCF-β-catenin transcriptional complex.

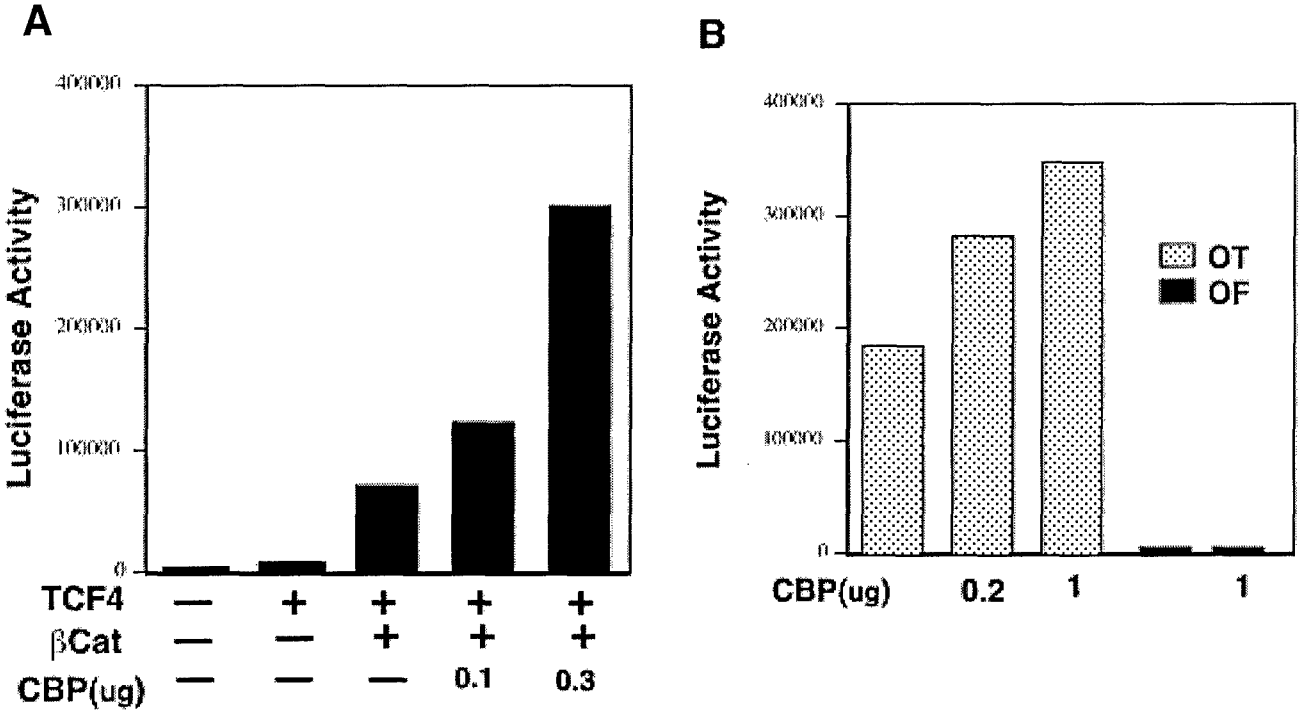

Figure 1. Co-expression of CBP potentiates TCF4-β-catenin transcriptional activity. (A) Expression plasmids for TCF4, stable form of -β-catenin (ΔN90) and CBP was

transfected into 293T cells alone or in combination as indicated. Note that CBP increases the TCF4-β-catenin dependent transcription activity in a dose-dependent manner. (B) Expression plasmids for TCF-responsive reporter (OT) or a mutant reporter (OF) was co-transfected with indicated amount of CBP. Note that only OT but not OF display high activity in response to CBP.

To further substantiate the positive effect of CBP on TCF4-β-catenin-dependent transcription, we inhibit CBP by the co-expression of adenovirus E1A, which binds CBP and inhibit its co-activation activity (3). As shown in Figure 2, consistent with the positive role of CBP in TCF4-β-catenin transcriptional activity, co-expression of E1A dominantly suppress the reporter activity. Supporting this conclusion, expression of E1A mutant that does not bind CBP (ΔN) fails to inhibit TCF-β-catenin mediated transcription. Based on these results, we conclude that CBP positively regulates TCF4-β-catenin transcriptional activity.

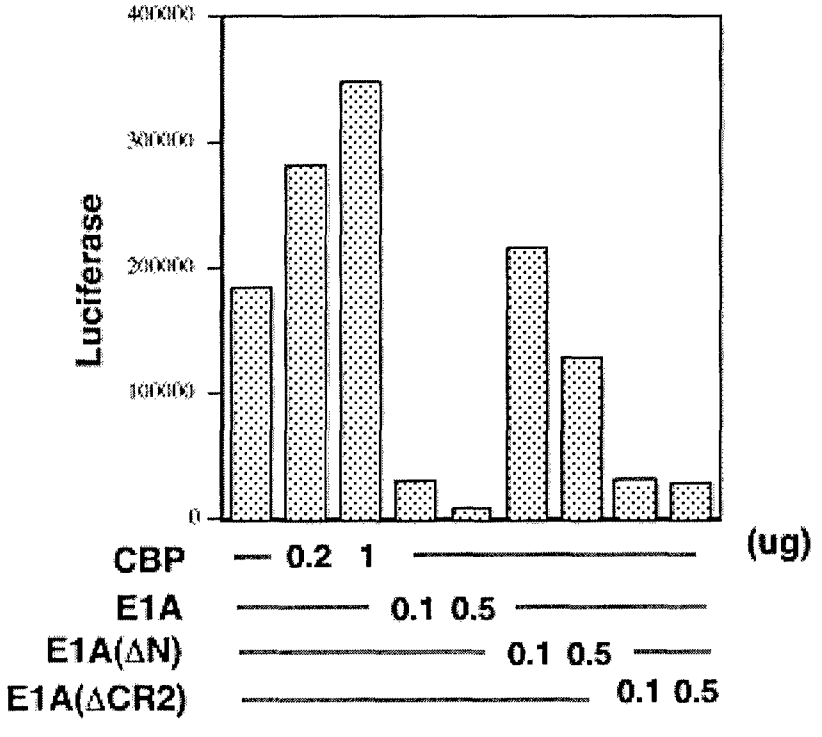

Figure 2. CBP binding protein E1A inhibits TCF-β-catenin mediated transcription. Expression plasmid for CBP, E1A, E1A mutant which can not bind CBP (ΔN) or pRB(CR2) were co-transfected into Sw480 cells as indicated. Note that wild type and CR2 mutant E1A but not CBP binding deficient (ΔN) mutant E1A can repress TCF-β-catenin dependent transcription.

To investigate how CBP might regulate TCF-β-catenin transcriptional activity, we investigate whether CBP forms a complex with TCF and/or β-catenin. As shown in figure 3, when the stable form of β-catenin (ΔN90) was co-expressed with CBP in 293T cells, a complex of β-catenin (ΔN90) and CBP can be detected. We note that a significant portion of ΔN90-β-catenin is localized in nuclei as judged by immunostaining on the transfected cells (data not shown), which allows its interaction with nuclear CBP.

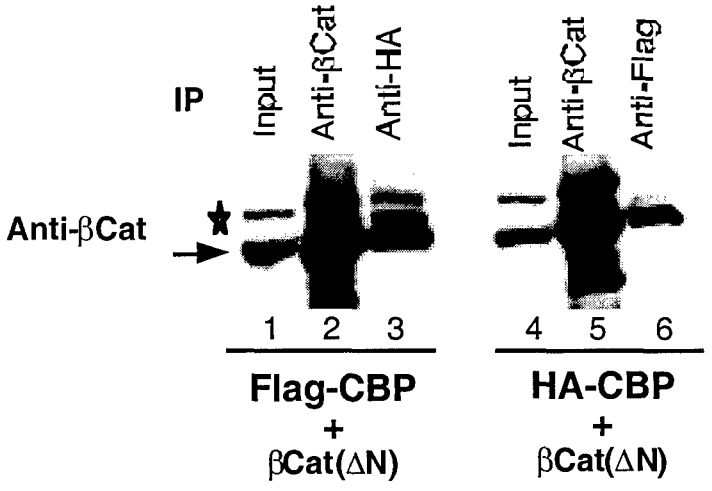

Figure 3. CBP interacts with -β-catenin. Expression plasmids for the stabilized form of β-catenin (ΔN90) and for either flag-tagged or HA-tagged CBP were transfected into 293T cells. Co-immunoprecipitation was performed using antibodies against HA (12CA5), Flag (M2) or β-catenin and then blotted with antibody against β-catenin. 2.5% of the total protein used in immunoprecipitation were loaded in the input lanes. We note that a ΔN90-β-catenin (arrows) was co-immunoprecipitated by both HA and Flag antibodies. The slower migrated band (asterisk) is the full-length endogenous β-catenin, which does not appear to interact with CBP strongly.

The results obtained so far shows that it is unlikely that CBP negatively regulates wnt signaling by suppressing TCF-β-catenin transcriptional activity. We thus consider the alternative hypothesis wherein CBP might potentiate TCF transcriptional repression. To test this idea, one need to first establish a TCF-dependent repression assay. However, in a typical transient transfection system, TCF-4 does not show any appreciable transcriptional repression activity (data not shown). One possibility for this observation might be due the nature of transient transfection. In the transient transfection system, the reporter gene is generally not properly assembled into chromatin-like structure, which might be essential for active transcriptional repression to occur. Indeed, it has been observed that TCF can only repress reporter gene that is stably integrated into chromosomes (H. Clevers, personal communication).

Although our initial attempt to address the role of CBP in TCF-mediated transcription repression is unsuccessful due to the assay limitation, we are now in the process of developing TCF mediated repression assay using properly chromatinized reporter template. To achieve this goal, we are taking two approaches. First, we will use Xenopous oocyte to perform the transcription repression assay. Reporter plasmids injected into oocyte have been shown to be quickly assembled into chromatin. Under this configuration, we have shown that the histone acetyltransferase activity (HAT) of CBP is specifically required for the transcription activity of nuclear hormone receptor (C.H. Lai, Z. Q. Huang, J.M.Wong and T.P.Yao, Manuscript in preparation). However, this activity is not required under the transient transfection system. We will use this system to determine if CBP is necessary for the active repression mediated by TCF. The second approach will utilize cell line stably expressing TCF reporter genes. TCF, CBP and -β-catenin will then be introduced by transient transfection and assess their functional status.

Key Research Accomplishment:

- We have uncovered an unexpectedly that CBP co-activates TCF-β-catenin dependent transcription, suggesting a dual role of CBP in modulating wnt signaling.

-CBP physically interacts with β-catenin. This observation provides a molecular mechanism explaining how the recruitment of β-catenin activates TCF-dependent transcription. This result suggests that tripartite complex of TCF-β-catenin –CBP is likely the functional transcriptional complex mediating wnt-dependent transcription.

Reportable Outcome:

Lai, C.-H, and Yao, T.-P. Transcriptional co-activator CBP interacts with β-catenin to control TCF transcriptional activity. (**In Preparation**).

Conclusion:

Our study has led to an unexpected observation that CBP plays a positive role in supporting the transcriptional activation potency of TCF. This is achieved by the physical interaction between CBP and β-catenin. Our observation provides two important insights into the wnt-signaling pathway. First, it provides a molecular basis on how the recruitment of β-catenin by TCF can activate transcription. Second, it suggests the possibility that CBP might play a dual role on modulating TCF activity. We hypothesize that in the absence of β-catenin, CBP functions to potentiate TCF transcriptional repression activity and the binding of β-catenin followed by Wnt signaling, coverts the CBP to a classical transcriptional co-activator that promotes TCF-β-catenin transcriptional activity (Figure 5). This hypothesis will explain all the existed genetic and molecular/biochemical data. We will examine this hypothesis in the second 12 months of the award period (see above for more experimental details).

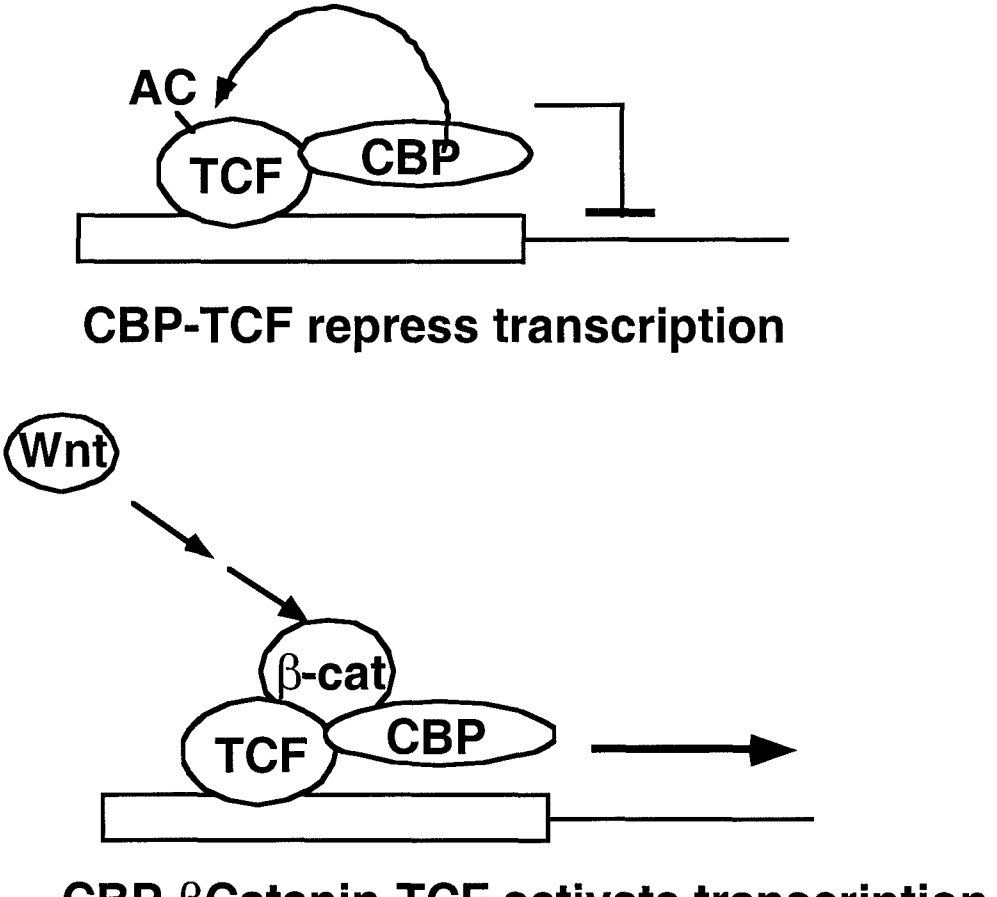

Figure 4. Model for the functional consequence of CBP-β-catenin-TCF interaction. In the absence of wnt signaling, CBP facilitates TCF to function as a transcriptional repressor, possibly by acetylating TCF. Upon wnt signaling, β-catenin is stabilized, translocates into nucleus and subsequently binds to CBP and TCF. The binding of β-catenin converts CBP to the transcriptional co-activator and consequently, TCF can now function as a transcriptional activator.

Reference:

1. Dierick, H. & Bejsovec, A. (1999) *Curr Top Dev Biol* **43,** 153-90.
2. Cavallo, R. A., Cox, R. T., Moline, M. M., Roose, J., Polevoy, G. A., Clevers, H., Peifer, M. & Bejsovec, A. (1998) *Nature* **395,** 604-8.
3. Arany, Z., Newsome, D., Oldread, E., Livingston, D. M. & Eckner, R. (1995) *Nature* **374,** 81-4.